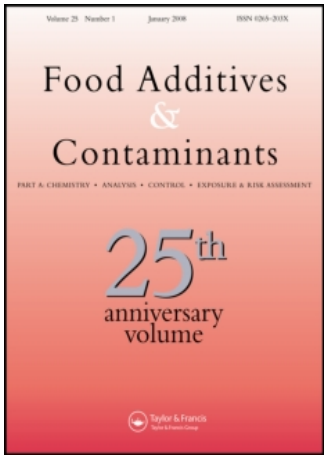

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Sample comminution for mycotoxin analysis: Dry milling or slurry mixing?

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Abstract
A comparison was made between dry milling and slurry mixing as a comminuting step preceding mycotoxin analysis. Sample schemes of up to 30 kg are mandated by European Commission legislation. Cocoa, green coffee, almonds and pistachio samples of 10 kg were milled by a Romer analytical sampling mill and all three subsamples were analysed for aflatoxin B_1 or ochratoxin A content. The homogenization process was evaluated in terms of the analytical results, coefficients of variation for different mills and particle size distributions. Coefficients of variation for the comminuting step were higher for dry milling than for slurry mixing. This difference was explained based on measured particle size distributions for both milling types. Measurements also showed slight differences in mycotoxin content of samples based on milling procedures. This might lead to lots being wrongly accepted or rejected based on an erroneous subsample result. It was concluded that sample comminution was best performed by slurry mixing, which produced smaller particles and, consequently, homogeneous samples with lowest coefficients of variation. Additional data are given on analytical results in 10-kg subsamples that originate from the aggregate 30-kg sample as described in Commission Directive 98/53/EC.

Keywords: *Subsampling, comminution, mycotoxin, slurry, dry-milling*

Introduction

Since 1 January 1999, European commission (EC) directives for aflatoxins have been strongly enforced, consisting of sampling plans that mandate sample weights of up to 30 kg. This raised questions about how these relatively large samples could fulfil the requirement to 'finely grind and mix thoroughly each laboratory sample using a process that has been demonstrated to achieve complete homogenization' (European Commission 1998). Large samples reduce the sampling error, i.e. the variance observed, when multiple samples are analysed. The disadvantage of large samples is the material handling, i.e. the homogenizing of large quantities of ground material. An aliquot (referred to as a subsample) must be taken from the homogenized sample. Here the determining factor for variance is the subsample size (or the weight of the solids portion of a slurry sample) as well as the particle size of the grind, as will be shown below. In the present discussion, the sample size will be emphasized, but subsample sizes will be indicated when available.

The critical points in the EC directive are the expressions 'grind finely' and 'mix thoroughly'. As such, these topics have been subject to several studies, but never up to the level of 30-kg sample sizes. Dickens and Satterwhite (1969) developed a mill that could handle peanut samples of up to 25 kg. They presented results of tests on 5-kg samples from which they withdrew 50-g subsamples, but gave no data on larger samples. Velasco and Morris (1976) considered the application of a water slurry to obtain finer particles and a more uniform particle distribution. Another advantage of slurry preparation is the avoidance of clogging of samples with a

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As per 2004, U. Jörissen retired from 'his' company, which is since become a member of the Eurofins group.
T. F. Schatzki retired from the institute in 2004.

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high oil content. They carried out experiments on different matrices with sample weights up to 4.5 kg (subsample sizes of 17–40 g), although they noted that the slurry preparation was limited only by the capacity of the equipment. Whitaker et al. (1980) considered a compromise. They prepared a slurry from a sample, which was first comminuted by another milling process. Due to the regulations of the USDA, they limited themselves to a sample size of only 1.1 kg (subsample 11 g). Despite this restriction, their method was developed into the alternative best foods method used for analysis of aflatoxin in peanuts (AOAC International 1998).

Dorner and Cole (1993) started all over again from the beginning with the 21.8-kg sample (100- and 200-g subsamples) of raw, shelled peanuts for analysis in official USDA-approved laboratories. They compared subsample variability between grinding with four different mills, but only with sample sizes up to 4 kg. Thus, the question of what the result would be on 21.8-kg samples (or other subsample sizes) remained unanswered. Their statistical data, especially variances, on the 2- and 4-kg samples were less favourable than data that can be achieved by applying slurry mixing. This may, of course, be due to sampling, rather than subsampling, variance. Scholten and Spanjer (1996) published data on slurry mixing for samples up to 10 kg, whereas the laboratory of Eurofins/Wiertz-Eggert-Jörissen had similar experiences, even when applying samples up to 30 kg. Calori-Domingues et al. (2000) evaluated variability for aflatoxin analysis in peanuts associated with sample preparation by dry milling with an RAS mill and slurry mixing with a blender. Unfortunately, however, they investigated only samples up to just 5 kg. Nor did they analyse all sample portions.

Following the work of Calori-Domingues et al. (2000), the laboratories of the Food and Consumer Protection Authority and of Eurofins/Wiertz-Eggert-Jörissen decided to perform new experiments with following goals: (1) what homogenization can be achieved when applying different milling processes for 10-kg samples?; and (2) what aflatoxin values are measured while doing so? This work is reported below. Concurrently, Schatzki and Toyofuku (2003) studied much the same questions for pistachios in some detail. They were the first to prove a relationship between particle size and subsampling variance for the slurry mixing process. Since their investigations provided the interpretation of the milling experiments, this lead to a joint presentation at the 2nd World Mycotoxin Forum, February 2003, the Netherlands (Spanjer et al. 2003), which is outlined in detail here. Mycotoxin data in all tables are expressed in $\mu g\,kg^{-1}$ and coefficients of variation are given as per cents.

Results of previous workers

The effect of grinding can depend on the matrix in which the contaminant of interest is present. The choice of matrices has been discussed at a CEN TC275/WG5 (Comité Européen de Normalization, Technical Committee 275, Working Group 5, Biotoxins. In this working group, representatives of European Union standardization bodies elaborate on method performance guidelines and draft methods of analysis for official food control as to harmonize at a European level) meeting, taking into account existing and upcoming legislation for different mycotoxins and food types. Combining both items leads to the conclusion that many matrices, existing as dried, whole or ground raw material, are to be considered. Also, differences in sample weight, i.e. between nuts and spices, exist. Suggestions for representative commodities were as follows:

- Cereals, since for this staple food directives exist on aflatoxins as well as on ochratoxin A and DON.
- Raisins, because these are included in directives for aflatoxins and ochratoxin A.
- Paprika powder as an example of an industrially ground commodity for which legislation on aflatoxins exists as well.

In practice, however, it turned out that the availability of naturally contaminated lots that could be used for these experiments was the limiting factor. The results presented here show what has been examined. Finally, the published results of previous workers suggested that the CV of subsampling was related to particle size: as expected, the smaller the particles, the smaller the resulting CV. A quantitative relation has now been derived by Schatzki and Toyofuku (2003) and will be shown below. It is, therefore, of some use to list these previous results to bring out this relationship. In chronological order, we start with the first results obtained with the subsampling mill of Dickens and Satterwhite (1969) for peanut kernels. In this mill the sample is simultaneously comminuted and subsampled. Their data are given in Table I. From these data, CVs can be calculated that vary from 9 to 43% in 5-kg samples with an aflatoxin B_1 content of 15–233 $\mu g\,kg^{-1}$. Their data on 500-g samples show that 11 of 18 have CVs below 10%, but these originate from just two data points and are given here for information only.

Velasco and Morris (1976) examined the application of water slurries for aflatoxin analysis. As can be seen in Table II, their results on 500- or 1000-g samples show lower CVs for all matrices, while analysing 16–40-g subsamples. They concluded

Table I. Aflatoxin content ($\mu g\,kg^{-1}$) of 50-g subsamples taken from 5 kg peanut samples ($n=4$) and 25-g subsamples taken from 500 g peanut samples ($n=2$) after subsampling by a Dickens mill (Dickens and Satterwhite 1969).

Sub 1	Sub 2	Sub 3	Sub 4	Mean	SD	CV	Sub 1	Sub 2	Mean	SD	CV
14	14	17	14	14.8	1.5	10.2	7.2	7.4	7.3	0.1	1.9
17	17	17	14	16.3	1.5	9.2	7.4	7.3	7.4	0.1	1.0
51	40	51	40	45.5	6.4	14.0	15.9	24.3	20.1	5.9	29.6
57	40	40	51	47.0	8.4	18.0	18.2	18.2	18.2	0.0	0.0
57	69	127	90	85.8	30.7	35.8	21.7	29.6	25.7	5.6	21.8
70	63	63	113	77.3	24.1	31.1	22.6	17	19.8	4.0	20.0
257	114	171	129	167.8	64.2	38.3	22.8	24.8	23.8	1.4	5.9
257	257	171	103	197.0	74.6	37.9	23.7	15.7	19.7	5.7	28.7
257	257	228	343	271.3	49.7	18.3	33.8	35.4	34.6	1.1	3.3
343	257	228	103	232.8	99.3	42.7	37.3	40.1	38.7	2.0	5.1
							39.2	39.2	39.2	0.0	0.0
							44.9	49.2	47.1	3.0	6.5
							57.9	44.5	51.2	9.5	18.5
							58.1	89	73.6	21.8	29.7
							64.8	65.9	65.4	0.8	1.2
							75.3	31.9	53.6	30.7	57.3
							89	90	89.5	0.7	0.8
							126	136.1	130.9	7.4	5.7

Table II. Comparison between a slurry preparation and dry milling ($n=5$) of several matrices by Velasco and Morris (1976).

Milling type	Mean		SD		CV	
Matrix	Slurry	Dry	Slurry	Dry	Slurry	Dry
Corn	49.8	49.6	1.3	3.8	2.6	7.6
Cottonseed	66.4	65.2	3	9.6	4.5	14.8
Cottonseed meal	75.3	71.9	3.4	4.1	4.5	5.7
Peanuts ($n=8$)	13.2		1.03		7.8	
Peanuts	48	40.9	2.5	8.5	5.2	20.8
Peanut butter	51.6	51.9	1.5	2.8	2.8	5.4
Peanut meal	63.6	52.6	2.8	5.5	4.4	10.5
Copra	49.8	53.4	2.2	4	4.4	7.5

that the use of water slurry reduces the variability because the distribution of particles is more uniformly achieved with slurry than with dry ground product. This conclusion was confirmed by Whitaker et al. (1980), who determined the particle distribution by measuring the percentage of material that passed successively smaller sieves. Their second conclusion is that seeds of high oil content are readily reduced to a fine particle size, whereas only a coarse grind is possible with conventional mills because of clogging. Their last conclusion was that the quantity of the water slurry that can be prepared is limited only by the capacity of the available blending or homogenizing equipment. Unfortunately, they did not prove the latter conclusion by some more experiments.

Dorner and Cole (1993) dealt with variability of dry milling between four mills. Twenty 2- and 4-kg samples of naturally contaminated peanuts were ground in a Dickens subsampling mill (DM), a Stephan model UM-12 vertical cutter mixer (SM), a Robot Coupe model RSI6Y-1 vertical cutter (RC1) and a Robot Coupe model R10P vertical cutter (RC2) mixer. In this respect, it has to be kept in mind that in the Stephan and Robot Coupe mixers the samples were only comminuted, and in the Dickens mill the sample was simultaneously comminuted and subsampled. Thus, from the other mixers the subsamples are taken manually. Figure 1 shows images of this equipment. From each 2-kg sample, ten 100-g subsamples and from each 4-kg sample, ten 200-g subsamples were withdrawn for analysis. The CV among each set of ten subsamples was determined for each mill. The results are summarized in Table III, which shows that the Dickens mill is less favourable for both sample sizes. The data of the Stephan vertical cutter mixer are comparable with data published by Whitaker et al. (1994) of a study on variability of 2.26-, 4.21- and 6.91-kg samples. From their tables 2 and 3, CVs can be calculated of 29.2 and 21.4% at total aflatoxin concentrations of 33.8 and 31.6 $\mu g\,kg^{-1}$, respectively.

Calori-Domingues et al. (2000) compared slurry making and dry milling of 5-kg samples. Nineteen 5-kg samples of naturally contaminated peanuts were prepared following two procedures: (1) dry grinding the peanuts in a Romer Analytical Sampling (RAS) mill and taking a 500-g subsample, and (2) preparation of slurry by mixing 4.5 kg ground peanut with water (1 : 1) in a blender. From each procedure, two portions were taken: one of 50 g from the RAS mill subsample and another of 100 g from the slurry. The variance among the contamination level of two portions withdrawn from the RAS mill subsample was twice the variance observed in the slurry subsamples. Therefore, it was concluded that the

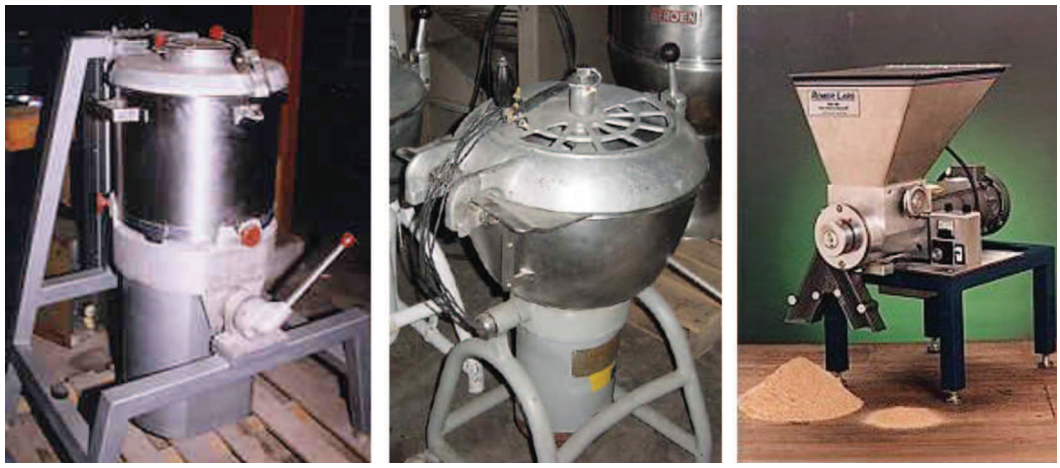

Figure 1. Hobart, Stephan and RAS mills.

Table III. Comparison of performances of four mills (Dorner and Cole 1993).

Sample size (kg)	Mill type	Average CV	Average total AF
2	DM	38.4	20.4
	SM	33.1	30.1
	RC1	20.1	32.9
4	DM	42.9	75.0
	SM	24.4	82.7
	RC2	24.3	69.6

slurry preparation procedure achieves better homogenization of the sample.

Schatzki and Toyofuku (2003) developed a theory for the subsampling CV, which could be written as:

$$CV_s = (Np_w s_w)^{-0.5}$$

to which an analytical CV should be added to obtain the total CV, where N is proportional to the subsample size; p_w is the average probability of finding a contaminated kernel in the lot, weighted by the concentration of such contamination; and s_w is inversely proportional to d_i^3, where d_i is the diameter of an individual particle i. p_w is heavily weighted towards high concentrations as well as proportional to average aflatoxin content. s_w is weighted towards large particles in the grind. While the factors on the right-hand side of this equation contain complex sums, they are easily evaluated once the pertinent distributions are known. p_w was obtained from previously measured aflatoxin distributions in a similar lot; s_w was measured by passing the subsample material through a set of screen sieves.

To test this expression experimentally, they dry ground a 10-kg sample of contaminated pistachios, added dry ice to avoid buttering, thoroughly mixed it and took twelve 20-g subsamples for which the aflatoxin B_1 was measured and CV computed.

Table IV. WRRC-ARS-USDA results on dry milling and slurry preparation (Schatzki and Toyofuku 2003).

Statistics	Dry grind	Water slurry
$\sum(w_i/s_i)$	0.00162	0.00034
Number of sub-particles g^{-1}	1233	5882
Weight average diameter (cm)	0.12	0.06
Probability of contamination p	0.00093	0.00122
Proportionality N	40	40
CV_s calculated	0.209	0.083
CV analytical	0.05	0.05
CV predicted (CV_s + CV analysed)	0.215	0.097
CV experimental	0.200	0.095
Mean ± standard error ($\mu g\,kg^{-1}$)	66 ± 4	87 ± 2

The remainder of the ground sample was mixed with (slightly less than) 15 litres water and slurry ground to a particle size about half that of dry ground material. In this way, the sample for all subsamples was the same and sampling variance does not come into play. Again, twelve 50-g subsamples (30 g water, 20 g pistachios) were taken and analysed. The results of Schatzki and Toyofuku (2003) are shown in Table IV, which shows that the calculated CVs matched the experimental ones within 0.015, for a theory that contains no adjustable parameters. They considered the theory proven. The means for dry grinding and wet slurry mixing differed by 31%. This effect is not understood at present. They also measured some other parameters such as milling times, slurry solvents and extraction solvent composition, but these are of lesser interest here.

Materials and methods

Apparatus

- Slurry mixer: Silverson type EX mixer (Silverson Machines Ltd, Waterside, Chesham, UK).

- RAS mill: Romer Analytical Sampling mill (Coring-System Diagnostix GmbH, Gernsheim, Germany).

Other laboratory equipment and slurry preparation procedures were as described previously (Scholten and Spanjer 1996; Schatzki and Toyofuku 2001). The RAS mill was applied according to the manual as supplied by the manufacturer (Release 2, January 1998). Before the dry milling process, the pistachio samples were frozen overnight at −20°C. The measurement of particle size distributions was described by Schatzki and Toyofuku (2003).

Reagents and materials

Aflatoxin measurements were performed as described by Stroka et al. (2000). Ochratoxin A measurements were carried out in cocoa (Entwisle et al. 2000) and in green coffee beans (Entwisle et al. 2001), including quality control. The only difference is that the fluorescence detection for Ochratoxin A was carried out as published by Zimmerli and Dick (1995).

Procedure. The experiments were carried out as follows:

- Sampling a lot according to the EC directive, resulting in a 10-kg sample.
- Milling the 10-kg sample by an RAS mill with a split ratio of 10%.
- Taking 50 g dry sample out of the 10% portion, as is usual for RAS mill users (subsample A).
- Slurry preparation of the remainder of the 10% portion of the sample (subsample B).
- Slurry mixing of the 90% portion by a Silverson mill (subsample C).
- Analysing the three subsamples A–C by HPLC methods.

Results and discussion

The results are given in the first three columns of Table V. They consist of measurements of Ochratoxin A in lots of cocoa and green coffee beans and of aflatoxin B_1 (the only aflatoxin of interest here) in lots of almonds, pistachios and mixed spices. The last two columns in Table V contain entries that can be calculated from the measured values. Explanations for these calculations are given below, but are included in Table V to facilitate any comparison of all the data. In the fourth column the mathematical mean of the three data points is presented, although it is based on only three data points per calculation, which on their turn originate from two different types of sample processing. In the fifth column, another sample value is shown. From the weight of each subsample and its mycotoxin content, it is possible to compute the best estimate for the mycotoxin content in the total sample. This calculated value is presented in the column 'weighted value'.

Table V. Measurements carried out according to the above procedure by Eurofins/Wiertz-Eggert-Jörissen (Hamburg, Germany) and the Food and Consumer Protection Authority (Amsterdam, the Netherlands).

	Sub A	Sub B	Sub C	A–C mean	Weighted value
Ochratoxin A matrix					
Cocoa	0.5	0.4	0.4	0.4	0.4
Cocoa	0.4	0.5	0.6	0.5	0.6
Cocoa	0.9	1.7	0.9	1.2	1.0
Cocoa	0.8	0.4	1.2	0.8	1.1
Cocoa	1.5	0.7	1.2	1.1	1.2
Cocoa	2.6	1.5	1.2	1.8	1.2
Cocoa	1.1	3	1.6	1.9	1.7
Cocoa	1.5	1.5	1.7	1.6	1.7
Cocoa	0.8	2.1	2.2	1.7	2.2
Cocoa	5.2	1.5	3.7	3.5	3.5
Cocoa	1.3	1.8	13	5.4	11.9
Green coffee	8.1	0.4	1.6	3.4	1.5
Green coffee	1.8	2.3	1.8	2.0	1.9
Green coffee	2.7	2.6	2.0	2.4	2.0
Green coffee	1.5	2.0	2.0	1.8	2.0
Aflatoxin B_1 matrix					
Almonds	1.0	0.2	2.2	1.1	2.0
Almonds	1.0	4.2	2.2	2.5	2.4
Almonds	0	0	3.4	1.1	3.1
Almonds	0.5	6.7	3.8	3.7	4.1
Mixed spices	4.2	8.1	7.8	6.7	7.8
Pistachio in shell	88.2	38	33	53.1	33.8
Pistachio in shell	51.4	42.4	44.2	46.0	44.1
Pistachio kernels	250	108	114	157.3	114.1
Pistachio kernels	204	122	126	150.7	126.0

Evaluation of the results must be done from the starting point of the experiments: milling the 10-kg sample by an RAS mill, which creates a division of the original sample in two subsamples of different weight. When RAS milling is used in daily routine analysis, this step is followed by taking an analytical sample out of the smallest subsample for further clean up and chemical analysis. In Table V, this situation is comparable with the results for subsample A in this experiment, with the crucial difference that data, as presented for subsamples B and C, are never measured in daily practice. In the case of sample preparation by means of slurry mixing, the whole sample is mixed into a slurry, and a portion is taken out of this slurry for further analysis. The best estimate of a measurement of these samples, as if they were handled as a whole by preparing a slurry, can be made by calculating the amount of mycotoxins from the individual A, B and C subsample values, taking their weight differences

into account. This calculated value is presented as 'weighted value' in Table V. On the other hand, the analytical values of subsamples A–C can be considered as three measurements in the same sample. But in that case, these three values originate from two milling processes. One is dry milling and for the second and third slurry mixing occurs after dry milling. The latter process will result in smaller particle sizes. Therefore, this mean is just given to get an idea of the order of magnitude.

When considering the effect of the milling process on the variability, it must be kept in mind what the consequences of an analytical result will be. The analytical results are crucial for the judgement on accepting or rejecting a lot. Aflatoxin B_1 is regulated in some EC regulations: $2\,\mu g\,kg^{-1}$ for nuts (EC 1525/98) and $5\,\mu g\,kg^{-1}$ for spices (EC 472/2002). For ochratoxin A only values from a working document (SANCO 2003) can be used: $2\,\mu g\,kg^{-1}$ for cocoa and $3\,\mu g\,kg^{-1}$ for green coffee beans. The latter values are under discussion and are only used here to evaluate the presented measurements. With these figures in mind, and without adding measurement uncertainties to the values in Table V, the differences between judgements of a lot based on dry milling (subsample A data) can be compared with the judgement of a lot that would have been obtained after slurry preparation of the sample as a whole, as indicated by the 'weighted value data' column in Table V. Doing so for ochratoxin A in cocoa, two of 11 lots would be rejected after a dry milling procedure, but three of these 11 lots after slurry preparation. In only one of these cases, the same lot would be rejected by both procedures. Thus, in two of the three cases the dry milling procedure would accept the lot that is rejected according to the weighted value and in one of these cases they differ even by a factor of 10. In one case, the dry milling procedure would reject a lot, which is accepted by the slurry preparation method. This would also happen with one of the four coffee samples. The same type of reasoning applies to the other commodities in Table V. The results for the measurements on aflatoxin B_1 are worse. In five of nine cases, the dry milling procedure would lead to acceptance of the lot, whereas the slurry preparation would reject eight of nine. A striking detail in this respect is the fact that in all five cases where the dry milling leads to acceptance of a lot, this happens at low levels, i.e. around the limit of the Directive. The aflatoxin levels in the pistachios are so high that these measurements lead to rejection in any case. If the overall results of Table V were considered, the dry milling procedure would reject seven of 24 lots, whereas the preparation of slurry would reject 11 of these 24 lots. Table VI gives an overview of this paragraph in numbers. It also reveals that dry milling would have lead to two false-positive results, one with cocoa and one with coffee beans. This is an interesting detail, since both commodities are rather expensive, so from this point of view even false-positive results are not desirable.

The different mycotoxin results for the different types of subsamples as compiled in Table V indicate that the homogenization procedure appears to have been incomplete. Bear in mind that in this paper, the analytical variance is considered to be negligible when compared with subsample variance and sampling variance is suppressed. Total variance is the sum of all three variances (Whitaker et al. 1974), of which only subsampling variance is investigated in this study. Due to the application of the slurry preparation to subsamples B and C, these two subsamples have been analysed in the same way. From Table V the differences can be seen easily by comparing the columns of A vs. B and A vs. C. The data on B and C are not available when dry milling is applied in daily routine analysis. For this investigation, these values were measured to be able to reconstitute the 'weighted value'. It is scientifically not correct to calculate the coefficient of variation of the dry milling process from these data, since the number of subsamples is only three and these originate from two different preparation procedures. For an impression of variances between different milling processes, only CVs can be obtained from literature, for which reason these were tabulated here. If one assumes the analytical error to be far less than any other error in any mycotoxin study in the past, one can focus on the published CV's as being caused mainly by subsampling.

For spices, only one data set is given in Table V. It was suggested that this matrix might be homogeneous after the industrial milling process. To examine this assumption, a supplier of spices provided the data sets presented in Table VII. After the industrial milling process of a lot, four incremental samples of 100 g were taken and analysed. The standard deviation of the analyte is low when compared with the mean, whereas the CVs can be very high, which is mainly due to the low level, which never exceeded the EC limits. The last test in Table VII was made on a sample of whole

Table VI. Overview of the rejection of lots and false-positive and -negative decisions.

Mycotoxin	OTA	AFB_1	Both
Sample *n*	15	9	24
Rejected by			
Dry	3	4	7
Slurry	3	8	11
Both	1	4	5
False-negative	2	4	6
False-positive	2	0	2

nutmeg nuts. As expected, when sampling nutmeg as whole nuts, the standard deviation and coefficient of variation were unacceptable.

To get an impression of the homogeneity that can be achieved when slurry mixing is used for sample preparation, data have been collected from different European Union laboratories that use this technique on a routine basis (Scholten and Spanjer 1996). They are shown in Table VIII. It demonstrates that CVs for routine nut samples are below 10% at aflatoxin B_1 levels down to $4\,\mu g\,kg^{-1}$. These data are comparable, if not even better, than the level Dorner (2002) achieved in his latest experiments, when he reported a CV = 3.6% at an aflatoxin content of $305\,\mu g\,kg^{-1}$, while applying water slurry to a 3.6-kg peanut sample in the same Stephan mill as used for the experiments in Table III. Unfortunately, he never tried bigger samples, whereas the Robot Coupe model R60, which has a capacity of 60 litres, would have been very suitable for such an experiment (to complete Table IX, its price is 22 k€).

Table VII. Aflatoxin B_1 in spices after industrial milling.

Spices	Sub 1	Sub 2	Sub 3	Sub 4	Mean	SD	CV
Paprika powder	0.8	1	1.2	0.9	1.0	0.2	17.5
	1.4	1.5	1.2	1.3	1.4	0.1	9.6
	1.3	1.6	1.4	1.2	1.4	0.2	12.4
	1.9	1.8	1.4	2.4	1.9	0.4	21.9
Curcuma	0.6	0.5	0.5	0.3	0.5	0.1	26.5
	0	0.6	0.5	0.6	0.4	0.3	67.6
Nutmeg	2.2	0.7	3.3	0.7	1.7	1.3	73.4
	0.6	1	1.1	1.7	1.1	0.5	41.3
	1.6	1.3	0.8	2.6	1.6	0.8	48.2
	1	1.3			1.2	0.2	18.4
Ginger	4.6	2.2			3.4	1.7	49.9
	1.7	2.4			2.1	0.5	24.1
Cayenne	2.1	2.9			2.5	0.6	22.6
	1.1	0.7			0.9	0.3	31.4
Nutmeg NUTS	0.1	0.5	1.7	39	14.9	26.6	178.0
(eight subsamples)	0.3	1	4.9	72			

Table VIII. Homogeneity in slurries of 7.5–30 kg nut samples.

Matrix	*n*	Mean	SD	CV	Member State
Pistachio	4	3.9	0.2	4.4	France
Pistachio	20	5.7	0.2	3.9	The Netherlands
Peanut	10	7	0.3	3.8	The Netherlands
Pistachio	4	7.5	0.6	8.2	France
Pistachio	10	10.4	1.0	9.7	Germany
Pistachio	20	10.9	0.5	4.5	The Netherlands
Pistachio	20	14.5	1.1	7.3	Austria

Data are from different Member States.

Apart from the analytical point of view, practical aspects of handling and maintenance of the mills should be considered. Table IX summarizes several items. The main problem for dry milling equipment is the clogging of matrix due to its oil content. The pistachio samples as described in Table V were deep-frozen (−20°C) before starting the milling process, yet they turned into a paste as well. Apart from the chances of diminishing homogeneity due to buttering, this necessitates more time for cleaning the mill after every sample. Schatzki and Toyofuku (2003) used dry ice (−80°C) when applying dry milling of his pistachio samples. But then evaporation to dryness of this dry ice takes time (they reported 90 min). Similar clogging and buttering problems occur when applying Stephan and Robot Coupe mills.

The milling minutes in Table IX are taken from the cited literature. Dickens and Satterwhite (1969) report a handling time of 7 min for a 5-kg sample including cleaning of the mill. Other references (Velasco and Morris 1976; Whitaker et al. 1980; Calori-Domingues 2000, Schatzki and Toyofuku 2001) report clogging, buttering and difficulties when removing the pasty sample after milling, since in those cases dismantling of the milling head will be required. This is a disadvantage for use in daily routine practice. Since the Dickens mill is a flow-through type mill, clogging also leads to blockage of the milling process. When applying slurry mixing, handling becomes very facile when the mill is mounted on a mobile hydraulic floor stand, which adds another 2 k€ to the equipment costs. With this accessory, it is possible to lift the mixing head hydraulically in and out of the slurry vessel, as shown in Figure 3. Variable mixing speed can be delivered

Table IX. Details on mills: price level autumn 2003 (k€ units, taxes excluded).

Mill, mark and type	Sample size (kg)	Milling (min)	Price (k€)	Remarks	Matrices
Dickens	25	11	n.a.	Not available; $3\,kg\,min^{-1}$	Peanut, brazil nut, soybean, corn
Stephan UM-12	6	7	4	Cleaning takes up to 10 min	Peanut
Robot Coupe RSI6Y-1	2.5	7	3	Cleaning takes up to 10 min	Peanut
Robot Coupe R10P	6	7	4	Cleaning takes up to 10 min	Peanut
Romer/RAS	10	6–9	8	Dismantling takes up to 10 min more time	Peanut, walnut, pistachio, almond, hazelnut
Silverson EX	10	5	6	Can handle up to 50 kg in 15 min milling time	Peanut, walnut, pistachio, almond, cocoa, corn, hazelnut

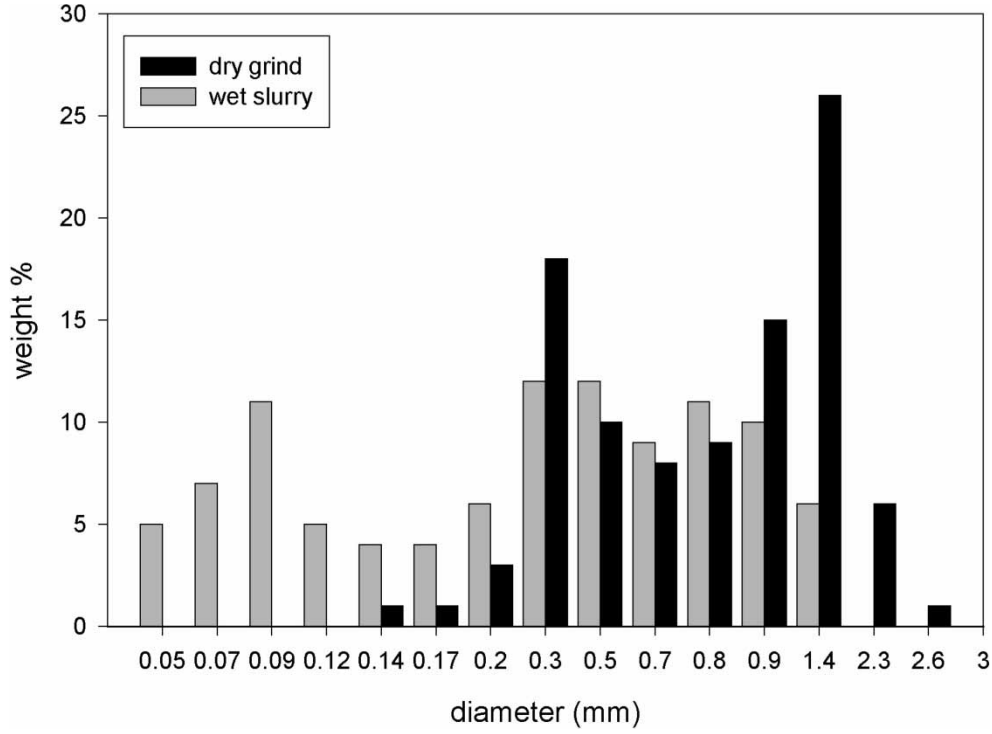

Figure 2. Particle size distributions of ground pistachio kernels (Schatzki and Toyofuku 2003).

Figure 3. Silverson mill hanging in floor stand and milling vessels, one of which is on wheels.

by a frequency inverter (4 k€). A complete system thus will cost 12 k€ (without VAT, which is 19% in the Netherlands) plus the cost of any required mixing vessels.

After mixing the slurry, the equipment can easily be cleaned by flushing it with a water showerhead. This way of handling also contributes to the safety of the analysts, since no exposure to dusty matrix occurs while mixing the sample. Dusty particles of the matrices may contain mycotoxins. In this respect, it has to be noticed that due to inhalation these particles pass immediately into the lungs. The only disadvantage of the slurry-making procedure is the amount of waste that has to be removed afterwards. Every 1 kg sample makes approximately 2–3 kg slurry, which has to be discharged according to the regulations for chemical waste disposal. The costs for this type of waste disposal is calculated to be some 0.1 €l^{-1} for the Amsterdam laboratory, in light of the fact that there is already a contract for all other waste disposal, which means that these are only additional costs. This means that waste disposal of a 10-kg sample that is diluted with 20 litres water adds €2 to the analyses costs. Compared with the total costs of a mycotoxin analysis this amount of money is rather low. Considering the ease of cleaning of the slurry mixer, this amount of extra costs is more than compensated by the savings on personnel time involved in dismantling, brushing and reconstructing dry milling equipment.

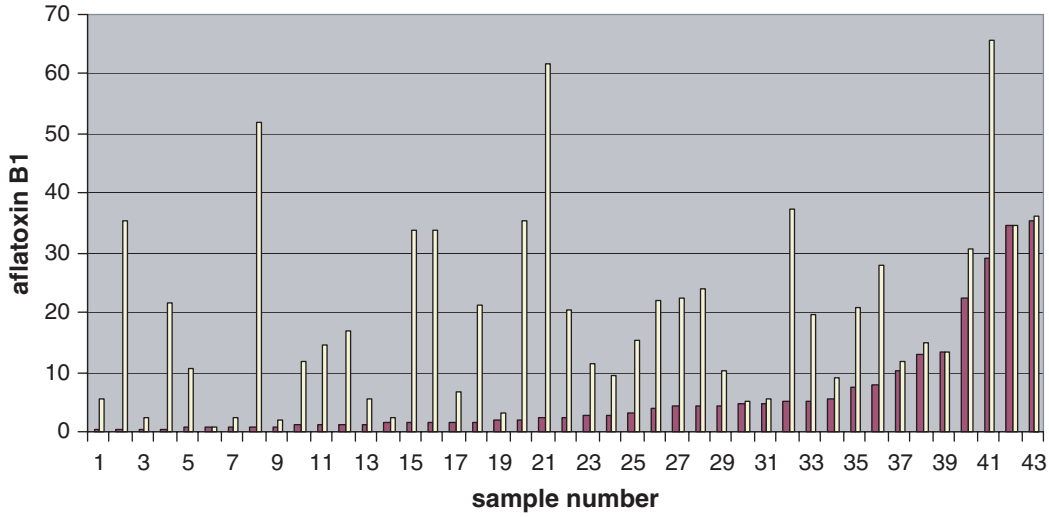

Figure 4. Aflatoxin B_1 content in two of three peanut subsamples of a lot.

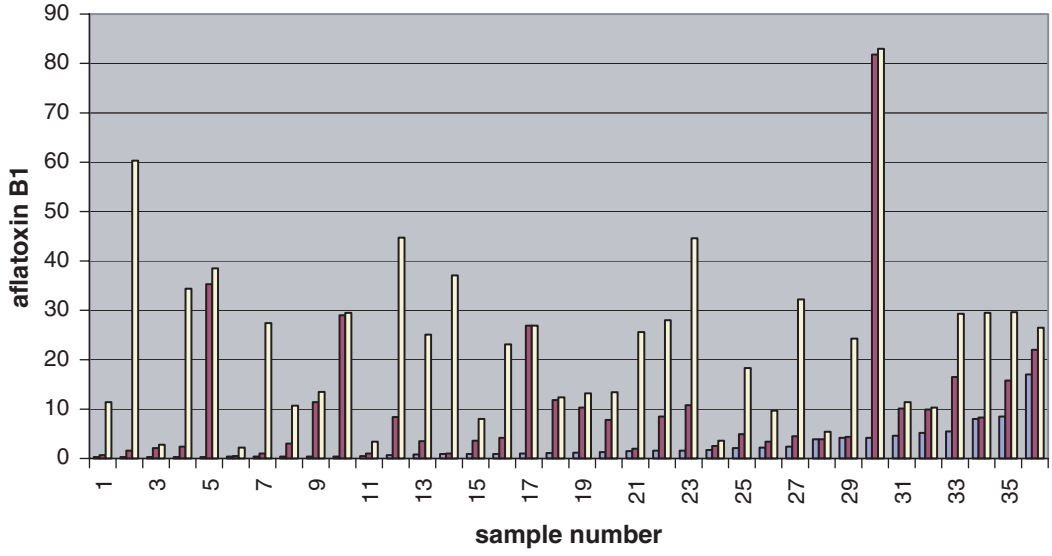

Figure 5. Aflatoxin B_1 content in all three peanut subsamples of a lot.

Slurry mixing is common practice at import control in the Netherlands for already many years. This means that a lot of analytical data are available on 3×10 kg subsamples of a lot, i.e. a container load of peanuts. Compiling data for 2001–04 revealed 111 data sets on peanuts only, of which following information could be derived:

- In 31 lots aflatoxin B_1 was found in only one subsample, ranging from 0.4 to 96.4 $\mu g\,kg^{-1}$.
- In 43 lots aflatoxin B_1 was found in two subsamples (Figure 4).
- In 37 lots aflatoxin B_1 was found in all three subsamples (Figure 5).

In Figure 4, the subsample data of the 37th lot, being 1.8, 2.9 and 228.1, are left out because they would upscale the figure in such a way that all other data would be too small to provide clear information.

Annex I of Commission Directive 98/53/EC notes that the aggregate sample of 30 kg 'has to be mixed and to be divided into three equal subsamples of 10 kg before grinding'. Due to the heterogeneity of the aflatoxin contamination and the possible high level in individual nuts, it can be expected that the measured aflatoxin levels in the three subsamples are not equal and even not at a comparable level. The data presented in Figures 4 and 5, and the ones mentioned above, clearly demonstrate that in practice it is possible to find up to almost 100 $\mu g\,kg^{-1}$ in one subsample and none in the accompanying other two subsamples. And if two subsamples contain aflatoxin, these can also be at the same level as being

as high as 62 $\mu g\,kg^{-1}$ in one subsample and 2 $\mu g\,kg^{-1}$ in the other. Similar observations can be made in Figure 5 where all three subsamples contain aflatoxin B_1. The cited directive also describes precisely that a lot has to be rejected if one of the subsamples exceeds the limit. The results reported here show that the criteria for accepting a lot need to be evaluated as well if 30-kg samples are mixed into one slurry and analysed as such instead of measuring three 10-kg slurries per lot.

Conclusions

Worldwide subsampling by dry milling is favoured because these mills are easy to apply and fast in comminuting samples into analytical portions. This might hold for samples up to 4 kg, but at a size of 10 kg nut samples suffer from clogging and buttering due to the high oil content. Slurry mixing is considered to be time consuming, in preparation and cleaning the equipment afterwards, what can be questioned when regarding the information presented in this article. If variability is evaluated, the available data are in favour of slurry mixing. Summarizing all the aspects, it can be concluded that slurry mixing can handle samples up to 10 kg in such a way that it leads to the lowest possible CVs and reveals the best estimate of the mycotoxin content of a lot. In this way, subsampling errors as well as chances on false-positive or -negative values, are reduced to a minimum. CVs for 30-kg samples that are slurry mixed as a whole give an indication that the same conclusion will also hold for these sample sizes. This could be confirmed by collecting some more homogenization data on 30-kg samples. Legal aspects on the rejection or acceptance of a lot have to be reconsidered when applying 30-kg slurry mixing.

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References

AOAC International 1998. In: Cunniff P, editor. Official methods of analysis, 16th ed., 4th revn, Vol. II. Gaithersburg, MD: AOAC International. Section 49.2.09, Method 970.45.

Calori-Domingues MA, Zovico C, Fonseca H, Gloria EM, Barbin D, Borguini RG. 2000. Variability associated with sample preparation procedures for aflatoxin analysis in peanuts. In: Sabino M, Rodriguez-Amaya D, Corrêa B, editors. Official program and abstract book of the Xth International IUPAC symposium on mycotoxins and phycotoxins. São Paulo: Instituto Adolfo Lutz. p 24.

Dickens JW, Satterwhite JB. 1969. Subsampling mill for peanut kernels. Food Technology 23:950–952.

Dorner JW. 2002. Simultaneous quantitation of Aspergillus flavus/A. parasiticus and aflatoxins in peanuts. Journal of AOAC International 85:911–916.

Dorner JW, Cole RJ. 1993. Variability among peanut subsamples prepared for aflatoxin analysis with four mills. Journal of AOAC International 76:983–987.

Entwisle AC, Williams AC, Mann PJ, Slack PT, Gilbert J. 2000. Liquid chromatographic method with immunoaffinity column cleanup for determination of Ochratoxin A in barley: Collaborative study. Journal of AOAC International 83: 1377–1383.

Entwisle AC, Williams AC, Mann PJ, Russell J, Slack PT, Gilbert J. 2001. Combined phenyl silane and immunoaffinity column cleanup with liquid chromatography for determination of ochratoxin A in roasted coffee: Collaborative study. Journal of AOAC International 84:444–450.

European Commission, Commission Directive 98/53/EC of 16 July 1998, laying down the sampling methods and the methods of analysis for the official control of the levels for certain contaminants in foodstuffs, Annex I, Sample preparation and criteria for methods of analysis used in official checking of the levels of aflatoxins in certain foodstuffs, 'Treatment of the sample as received in the laboratory, finely grind and mix thoroughly each laboratory sample using a process that has been demonstrated to achieve complete homogenisation'. Brussels: European Commission.

SANCO 2003. SANCO/3352/99-PARTIM, Working Document. Brussels: European Community. This does not necessarily represent views of commission services. Latest update SANCO/0063/2003-rev. 1. This version was valid while carrying out these experiments. The document has been updated several times since then, latest in February 2005.

Schatzki TF, Toyofuku N. 2001. Subsample preparation of pistachios. In: Proceedings of the 6th International Symposium on Fruit, Nut and Vegetable Process Engineering, Potsdam, Germany, 12 September 2001.

Schatzki TF, Toyofuku N. 2003. Sample preparation and presampling of pistachios. Journal of Agricultural and Food Chemistry 51:6068–6072.

Scholten JM, Spanjer MC. 1996. Determination of aflatoxin B_1 in pistachio kernels and shells. Journal of AOAC International 79:1360–1364.

Spanjer MC, Kastrup S, Schatzki TF. 2003. Sample preparation: slurry mixing or dry milling? Poster presentation at the 2nd World Mycotoxin Forum, Noordwijk, the Netherlands.

Stroka J, Anklam E, Jörissen U, Gilbert J. 2000. Immunoaffinity column cleanup with liquid chromatography using post-column bromination for determination of aflatoxins in peanut butter,

pistachio paste, fig paste and paprika powder: Collaborative study. Journal of AOAC International 83:320–340.

Velasco J, Morris SL. 1976. Use of water slurries in aflatoxin analysis. Journal of Agricultural and Food Chemistry 24:86–88.

Whitaker TB, Dickens JW, Monroe RJ. 1974. Variability of aflatoxin test results. Journal of the American Oil Chemists' Society 51:214–218.

Whitaker TB, Dickens JW, Monroe RJ. 1980. A water slurry method of extracting aflatoxin from peanuts. Journal of the American Oil Chemists' Society 57:269–272.

Whitaker TB, Dowell FE, Hagler Jr, WM, Giesbrecht FG, Wu J. 1994. Variability associated with sampling, sample preparation and chemical testing for aflatoxin in farmers' stock peanuts. Journal of AOAC International 77:107–116.

Zimmerli B, Dick R. 1995. Determination of Ochratoxin A at the ppt level in human blood, serum, milk and some foodstuffs by high-performance liquid chromatography with enhanced fluorescence detection and immunoaffinity column cleanup: Methodology and Swiss data. Journal of Chromatography B 666:85–99.